

IN THE CLAIMS

This listing of claims replaces all prior versions, and listings, in this application.

1. (previously presented) A biosensor for ligand, which comprises a bacterial periplasmic binding protein (bPBP) which is not glucose binding protein or maltose binding protein, and at least one reporter group attached at one or more amino acid positions of said bPBP which is an allosteric or endosteric site; wherein binding of said ligand in a ligand-binding pocket of said biosensor causes a change in signaling by said reporter group.
2. (original) The biosensor according to claim 1, wherein said bPBP is selected from the group consisting of arabinose binding protein (ABP), ribose binding protein (RBP), dipeptide binding protein (DBP), glutamine binding protein (QBP), histidine binding protein (HBP), glutamate/aspartate binding protein (EBP), phosphate binding protein (PBP), sulfate binding protein (SBP), and Fe(III) binding protein (FeBP).
3. (previously presented) The biosensor according to claim 1, wherein said bPBP is comprised of one or more mutations at an amino acid position(s) where said reporting group is not covalently linked.
4. (original) The biosensor according to claim 1, wherein said ligand-binding pocket is comprised of one or more mutations and said ligand does not bind to wild-type bPBP.
5. (previously presented) The biosensor according to claim 1, wherein said reporter group is attached to said bPBP at one or more amino acids positions distal from said ligand-binding pocket.
6. (previously presented) The biosensor according to claim 1, wherein said reporter group is attached to said bPBP at one or more amino acid positions proximal to said ligand-binding pocket.

7. (previously presented) The biosensor according to claim 1, wherein said reporter group is covalently attached at one or more amino acid positions of said bPBP.
8. (previously presented) The biosensor according to claim 1, wherein said reporter group is noncovalently attached at one or more amino acid positions of said bPBP.
9. (original) The biosensor according to claim 1, wherein said reporter group is a redox cofactor.
10. (original) The biosensor according to claim 1, wherein said reporter group is a fluorophore.
11. (original) The biosensor according to claim 1, wherein said biosensor's standard intensity change (ΔI_{std}) upon binding of ligand is greater than 0.25.
12. (original) The biosensor according to claim 11, wherein said ΔI_{std} is greater than 0.9.
13. (original) The biosensor according to claim 1, wherein said biosensor's maximum value of standard ratiometric change (ΔR_{max}) upon binding of ligand is greater than 1.25.
14. (original) The biosensor according to claim 13, wherein said ΔR_{max} is greater than 2.5.
15. (previously presented) A biosensor for ligand, which comprises a bacterial periplasmic binding protein (bPBP) and at least one reporter group attached at one or more amino acid positions of said bPBP which is an endosteric site.
16. (withdrawn) A method of detecting presence or absence of ligand in a sample, which comprises: contacting a biosensor according to claim 1 with said sample under conditions such that said biosensor is able to bind to ligand present in said sample;

comparing the signal transduced by said reporter group when said biosensor is contacted with said sample with the signal(s) transduced by said reporter group when said biosensor is contacted with at least one control sample containing a known quantity of ligand; and determining the presence or absence of ligand in said sample from said comparison.

17. (withdrawn) A method of quantitating amount or concentration of ligand in a sample, which comprises: contacting a biosensor according to claim 1 with said sample under conditions such that said biosensor is able to bind to ligand present in said sample; comparing the signal transduced by said reporter group when said biosensor is contacted with said sample against signals transduced by a series of control samples containing known quantities of ligand; and calculating the quantity of ligand in said sample from said comparison.

18. (withdrawn) A method of assaying for ligand in a sample, which comprises:

- (a) contacting a biosensor according to claim 1 with said sample, wherein binding of said ligand in a ligand-binding pocket of said biosensor causes a change in signaling by said reporter group;
- (b) measuring a ratiometric change (ΔR) for the signal transduced by said reporter group; and
- (c) at least detecting or quantitating ligand present in said sample.

19. (withdrawn) The method of claim 18, wherein said sample is comprised of a physiological fluid.

20. (withdrawn) The method of claim 19, wherein said physiological fluid is selected from the group consisting of blood, interstitial fluid, lavage, perspiration, plasma, saliva, serum, and urine.

21. (withdrawn) The method of claim 18, wherein said sample is suspected of being comprised of a ligand selected from the group consisting of amino acids, bioactive solid

and gaseous compounds which are soluble, carbohydrates, contraband or controlled substances, environmental pollutants, explosives, food contaminants and byproducts, lipids, metal ions, microbial toxins, neurotransmitters, nucleosides or nucleotides, peptides, steroids, and therapeutic drugs.

22. (withdrawn) A method of constructing a biosensor, which comprises:

- (a) selecting a first bacterial periplasmic binding protein (bPBP) of known amino acid sequence, wherein said first bPBP's three-dimensional structure has not been solved;
- (b) modeling said first bPBP's three-dimensional structure on a three-dimensional structure which has been solved for a second bPBP;
- (c) aligning at least one allosteric, endosteric, or peristeric site of said second bPBP with one or more putative amino acid positions at which reporter groups are to be attached to said first bPBP; and
- (d) attaching at least one reporter group at one or more of said putative amino acid positions of said first bPBP to form a putative biosensor and determining whether said putative biosensor is functional.

23. (previously presented) A method of constructing a biosensor, which comprises attaching at least one reporter group at one or more amino acid positions of a bacterial periplasmic binding protein (bPBP) which is an allosteric or endosteric site to form said biosensor, wherein binding of said ligand in a ligand-binding pocket of said biosensor causes a change in signaling by said reporter group and said bPBP is not glucose binding protein or maltose binding protein.

24. (previously presented) A method of detecting presence or absence of ligand in a sample, which comprises: contacting a biosensor according to claim 15 with said sample under conditions such that said biosensor is able to bind to ligand present in said sample; comparing the signal transduced by said reporter group when said biosensor is contacted with said sample with the signal(s) transduced by said reporter group when said biosensor is contacted with at least one control sample containing a

known quantity of ligand; and determining the presence or absence of ligand in said sample from said comparison.

25. (previously presented) A method of quantitating amount or concentration of ligand in a sample, which comprises: contacting a biosensor according to claim 15 with said sample under conditions such that said biosensor is able to bind to ligand present in said sample; comparing the signal transduced by said reporter group when said biosensor is contacted with said sample against signals transduced by a series of control samples containing known quantities of ligand; and calculating the quantity of ligand in said sample from said comparison.

26. (previously presented) A method of assaying for ligand in a sample, which comprises:

- (a) contacting a biosensor according to claim 15 with said sample, wherein binding of said ligand in a ligand-binding pocket of said biosensor causes a change in signaling by said reporter group;
- (b) measuring a ratiometric change (ΔR) for the signal transduced by said reporter group; and
- (c) at least detecting or quantitating ligand present in said sample.

27. (previously presented) The method of claim 26, wherein said sample is comprised of a physiological fluid.

28. (previously presented) The method of claim 27, wherein said physiological fluid is selected from the group consisting of blood, interstitial fluid, lavage, perspiration, plasma, saliva, serum, and urine.

29. (previously presented) The method of claim 26, wherein said sample is suspected of being comprised of a ligand selected from the group consisting of amino acids, bioactive solid and gaseous compounds which are soluble, carbohydrates, contraband or controlled substances, environmental pollutants, explosives, food contaminants and

byproducts, lipids, metal ions, microbial toxins, neurotransmitters, nucleosides or nucleotides, peptides, steroids, and therapeutic drugs.

30. (previously presented) A method of constructing a biosensor, which comprises attaching at least one reporter group at one or more amino acid positions of a bacterial periplasmic binding protein (bPBP) which is an endosteric site to form said biosensor.